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# Diurnal patterns and relationships between physiological and self-reported stress in patients with epilepsy and psychogenic non-epileptic seizures

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## **Abstract**

*Purpose:* Patients with epilepsy and those with psychogenic non-epileptic seizures (PNES) experience high levels of stress and stress is one of the most frequently self-identified seizure precipitants. Although stress is a multifaceted phenomenon, few studies have systematically examined its different components in patients with seizures. The aim of this study was therefore to describe diurnal patterns of psychological and physiological measures of stress in patients with epilepsy and patients with PNES, and explore their relationships to each other in order to improve our understanding of the mechanisms underlying stress and seizure occurrence in these patients.

*Method:* A range of stress markers including self-reported stress, salivary cortisol and heart rate variability (HRV) were explored in adult patients with refractory epilepsy ( $N = 22$ ) and those with PNES ( $N = 23$ ) undergoing three- to five-day video-telemetry.

*Results:* A diurnal pattern was observed in the physiological measures, characterised by higher levels of physiological arousal in the mornings and lower levels at night in both patients with epilepsy and PNES. The physiological measures (cortisol and HRV) were associated with each other in patients with epilepsy, no close relationship was found with self-reported stress in either of the two patient groups.

*Conclusion:* The findings contribute to and expand on previous studies of the patterns of stress in patients with seizures. The results also indicate a discrepancy between patients' physiological responses and their subjective stress perceptions, suggesting that simple self-reports cannot be used as a proxy of physiological arousal in patients with seizures and stress. Stress in these patient groups should be studied using a combination of complementary measures.

**Keywords:** epilepsy; psychogenic non-epileptic seizures; psychological stress; physiological stress; circadian rhythms

## 1. Introduction

Epilepsy, characterised by recurrent seizures, is one of the commonest disabling neurological disorders [1]. Long-term antiepileptic drug treatment controls seizures in 60 - 65% of patients; however, about one third of patients do not respond to medication [2]. Unless seizures are controlled altogether, patients' quality of life is related less to the frequency and severity of seizures than to psychosocial factors, such as social isolation, depression and anxiety [3].

Psychogenic non-epileptic seizures (PNES) are episodes of involuntary alteration of consciousness and disturbance of motor, sensory, autonomic or cognitive functioning that superficially resemble epileptic seizures but that are not caused by epileptic activity in the brain [4]. PNES are interpreted as an experiential and behavioural reaction to arousal triggered by internal or external stimuli [5]. 10 - 20% of patients newly presenting in seizure clinics with transient loss of consciousness have PNES [6]. Most patients with PNES meet the diagnostic criteria of conversion or functional neurological symptom disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) [7] and dissociative disorder in the International Classification of Diseases (ICD-10) [8].

High levels of self-perceived stress have been found in both epilepsy and PNES [9, 10]. This may be due to elevated rates of psychiatric comorbidities such as depression and anxiety and the disabling effects of living with a chronic condition, but may also be related to the experience of recurrent ictal events and the significant physiological arousal associated with epileptic or non-epileptic seizures [11-14]. Many studies have demonstrated that patients with epilepsy report stress as the commonest trigger of their seizures [15], and it is biologically plausible that the pathophysiological effects of stress on the neuroendocrine and immune systems contribute to the development and exacerbation of epilepsy [16]. Stress should be even more relevant in PNES, if PNES are a behavioural or dissociative response to emotional, physiological or social distressing triggers [17, 18].

Stress is a complex and multifaceted phenomenon comprising a range of interacting autonomic, endocrine, immune, cognitive, affective and behavioural processes. The physiological stress response is mediated by the neuroendocrine system, with its two main components, the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympathetic-adrenomedullary (SAM) system [19]. The activity of the HPA axis is characterised by a hormonal response in which the hypothalamus increases secretion of corticotropin-releasing hormone (CRH), causing the anterior pituitary gland to release adrenocorticotrophic hormone (ACTH), which activates the adrenal cortex to produce corticosteroid hormones (cortisol in humans, corticosterone in animals). Cortisol levels are one of the most frequently used markers of acute stress reactivity as well as a measure of exposure to long-term stress [20]. Cortisol secretion has a well-established circadian pattern, with circulating levels of cortisol typically highest within one hour of awakening and declining throughout the day to reach very low or undetectable levels around night-time sleep onset. Cortisol levels then begin to increase again between 2.00 and 4.00am [21]. Long-term exposure to stressors is associated with a chronic elevation of cortisol and a diminution of this natural diurnal fluctuation [22, 23]. Disruption of the circadian cortisol rhythm has also been found in conditions such as depression and chronic fatigue, and is associated with increased cardiovascular risk [21, 22].

The effects of corticosteroid hormones on seizure occurrence have mostly been investigated in animal models, suggesting that physiological stress as reflected by elevated corticosteroid

levels may exacerbate epileptiform activity (or its effects) in the brain [15, 24]. There is only limited evidence about the interactions between epilepsy and the circadian rhythms of cortisol. A number of studies have investigated cortisol levels in patients with epilepsy and found elevated cortisol levels post-ictally but no differences in baseline levels between patients and healthy controls [25].

There is some evidence suggesting the involvement of the HPA axis in the pathology of PNES. Patients with PNES, especially those with a history of sexual abuse, have been found to have higher levels of cortisol at baseline than healthy individuals [26]. Cortisol levels of patients with PNES were also elevated in a study of automatic avoidance [27]. Furthermore, levels of baseline cortisol in patients with PNES were positively correlated with an attentional bias to threatening social stimuli [28].

The SAM system responds to stress via the two branches of the autonomic nervous system (ANS), the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) that regulate the homeostatic function of the organism by a mutually antagonistic influence on internal organs [29]. Exposure to a stressful stimulus activates the SNS via release of adrenalin and noradrenalin, causing physiological arousal. PNS activation, mediated mainly by the vagus nerve, is responsible for on-going regulatory and feedback processes. In the initial response to stress, PNS tone is decreased whilst the most appropriate response to a particular stressor is selected. Because of its accessibility and high temporal resolution, heart rate variability (HRV) is an increasingly utilised marker of ANS activity. HRV reflects the dynamic influences of PNS and SNS tone on the heart and can be obtained non-invasively from electro-cardiographic (ECG) recordings.

Several studies reported HRV alterations in patients with epilepsy. Compared to healthy controls patients with epilepsy (especially when refractory) have been found to have reduced vagal tone both ictally and interictally [12, 30-34]. However, few researchers have examined the diurnal patterns of HRV in patients with epilepsy. One study found suppressed circadian HRV characterised by attenuation of the normal night time increase of HRV in patients with temporal lobe epilepsy, compared to healthy controls [35].

The evidence is even more limited for patients with PNES. The previously mentioned study of automatic avoidance demonstrated reduced HRV in patients with PNES at rest [26]. HRV was also significantly lower in patients with PNES compared to healthy controls in a study of attentional biases [36]. In addition, Ponnusamy and colleagues showed that resting HRV is reduced in both patients with epilepsy and PNES [37].

There is a lack of studies measuring the different modalities of stress in combination or exploring how different physiological and psychological stress measures relate to each other in patients with seizures. Better knowledge of these relationships would be valuable and improve our understanding of the mechanisms underlying stress and seizure occurrence in these patients. This could have useful implications for seizure detection or forecasting as well as the design of therapeutic interventions to reduce stress or seizures. The current study was intended to explore the patterns of physiological and self-reported stress measures across the day and the interrelationships between the different measures.

## 2. **Methods**

### 2.1 ***Participants and Design***

This study prospectively assessed daily levels of physiological and psychological stress in consecutive patients undergoing three- to five-day in-patient video-electroencephalographic / electrocardiographic (video-EEG/ECG) monitoring. We recruited adult patients with refractory seizures (epilepsy or PNES) admitted for diagnostic or pre-surgical video-EEG/ECG monitoring at the Royal Hallamshire Hospital in Sheffield. The diagnosis (epilepsy or PNES) was confirmed by the analysis of the video-EEG recording of at least one typical seizure by a trained Neurophysiologist. When a seizure had been recorded, the patients' consultant was asked to confirm that the recorded seizure was typical of habitual events and that it matched the patients' ultimate diagnosis, taking account of all clinical information available. Where no typical seizure was recorded, diagnoses were established clinically on the basis of a case review by two consultants specialised in epilepsy, taking account of all available clinical information. All patients in the PNES group had seizures involving impairment of awareness or responsivity (i.e. episodes which could not have represented misclassified panic attacks). Patients whose diagnosis remained uncertain or patients thought to have mixed disorders with epileptic and non-epileptic seizures were excluded from further analyses. The study received ethical approval by the North East Research Ethics Committee Yorkshire & The Humber (August 2013).

### 2.2 ***Outcome Measures***

#### 2.2.1 ***Baseline measures***

***Perceived Stress Scale – 4 Items*** [38] is a short scale developed to measure the degree to which situations in people's lives are perceived as stressful. The scale is a global measure of non-specific stress over the course of the past month, rated on a 5-point scale. The PSS-4 has been used and validated in patients with epilepsy [39, 40]. A previous longitudinal study by Thapar et al. used the PSS-4 to measure stress in a large cohort of patients with epilepsy to examine whether stress, anxiety and depression predict seizure frequency and seizure recency [40]. The mean baseline PSS-4 score in this study was 5.43 (SD = 3.56). The PSS-4 scale demonstrated good internal consistency in the present sample (4 items;  $\alpha = 0.79$ ).

***Liverpool Seizure Severity Scale – Revised*** [41] is a 12-item inventory designed to quantify the severity of patient's seizures. It provides a single-unit weighted scale that measures the severity of the severest seizures the patient has experienced during the past four weeks. The internal consistency in our sample was good (12 items;  $\alpha = 0.85$ ).

***Neurological Disorders Depression Inventory for Epilepsy*** [42] is a 6-item inventory developed to detect depression in patients with epilepsy by assessing common symptoms of depression experienced in the past two weeks that can be differentiated from adverse effects of anti-epileptic drugs. A score of more than 15 on the NDDI-E has 90% specificity and 81% sensitivity for a diagnosis of major depression [42]. The inventory had good internal consistency in this sample (6 items;  $\alpha = 0.80$ ).

***Generalised Anxiety Disorder 7-item Scale*** [43] assesses anxiety symptoms experienced over the course of the past two weeks. The GAD-7 has previously been used as a screening tool in epilepsy [44]. The internal consistency in this sample was excellent (7 items;  $\alpha = 0.91$ ).

**Quality of Life in Newly Diagnosed Epilepsy - 6 Dimensions** [45] was used as an epilepsy-specific measure of the health-related quality of life (HRQoL). The NEWQOL-6D assesses the HRQoL on six dimensions, including worry about attacks, depression, memory, concentration, control, and stigma. A single utility value between 0 (poor health) and 1 (perfect health) can be derived based on a formula developed by the authors of the scale [46]. The internal consistency reliability in this sample was acceptable (6 items;  $\alpha = 0.62$ ).

**Life-Orientation Test – Revised** [47] is a 6-item scale designed to assess generalized optimism. The LOT-R has been used in a number of behavioural, affective and health-related studies (for a review, see [48]). The internal consistency reliability in the sample was good (6 items;  $\alpha = 0.83$ ).

**Single-Item Self-Esteem Scale** [49] is a single-item measure of global self-esteem, scored on a 5-point Likert. Test-retest reliability and construct validity has been demonstrated in a number of studies [49].

**Brief Resilient Coping Scale** [50] is a 4-item measure designed to assess resilient coping, or tendencies to cope with stressful situations in an adaptive manner. The internal consistency reliability in this study was good (4 items;  $\alpha = 0.79$ ).

## **2.2.2 Daily measures**

### **Smith Stress Symptoms Inventory [51]**

The SSSI is a 35-item measure of commonly reported stress symptoms, comprising 6 sub-scales encompassing different symptom categories, including worry/negative emotion, attentional deficits, striated muscle tension, autonomic arousal/anxiety, depression, and interpersonal conflict/anger. A total score can be calculated from all 35 items to indicate overall level of stress symptomatology. A version of the scale assessing current stress symptoms (“right now at the present moment”) was used in this study. The consistency reliability of the SSSI was excellent (35 items;  $\alpha = 0.97$ ).

### **Heart rate variability**

Time-domain HRV parameters representing direct statistical or geometric measures of the beat-to-beat or inter-beat intervals were used in this study, including the standard deviation of all NN intervals (SDNN) and the square root of the mean of the sum of squares of differences between adjacent NN intervals (RMSSD). Non-linear HRV metrics including the cardiovagal index (CVI) as a measure of PNS activity, and cardiosympathetic index (CSI) as a measure of the SNS tone were also calculated [52]. For the analysis of HRV in this study we selected three- to ten-minute samples of resting ECG free of muscle artefact or ectopic beats.

### **Salivary cortisol**

Salivary cortisol is one of the most informative markers of the stress response, accurately reflecting the levels of cortisol in the blood [28, 53, 54]. In this study, saliva was sampled each morning at approximately 9am and each evening at 10pm using Salivette collection tubes (Sarstedt Ltd). Cortisol levels were assessed separately in the morning and evening, and the diurnal pattern was also explored by examining cortisol deltas (the difference between the morning and evening cortisol). Factors that influence levels of cortisol, including smoking, food intake or consumption of drinks with low pH were controlled as part of the testing procedure. The samples were analysed using liquid chromatography and tandem mass spectrometry (LC-MS/MS) [55, 56].

### **2.3 Procedure**

Patients who provided informed consent to take part in the study were asked to complete the baseline questionnaires. Participants were then asked to follow the same daily stress data collection procedure every evening at 10pm and every morning at 9am. They were asked first to complete the daily Smith Stress Symptom Inventory, then lie down in a supine position and rest without moving for ten minutes while breathing normally, in order to obtain a resting ECG recording for the extraction of HRV parameters, free of movement artefact and with a constant respiration rate. Saliva was sampled after this rest period. Participants were instructed not to smoke or eat or to drink anything but water for one hour before taking the saliva sample. The video-EEG/ECG recordings were consulted to check the fidelity to the testing procedure. All measures were taken at least 20 minutes before or after an occurrence of a seizure.

### **2.4 Data Preparation**

Patients' video-EEG/ECG recordings were reviewed using the XLTEK EEG software. Where the video recording revealed that the participant failed to complete the questionnaires or take the saliva sample on the given day or did so more than two hours before or after the specified time, the data were excluded from the analysis. Saliva samples were also excluded if the recordings showed that the participant consumed food or drinks less than 30 minutes before taking the saliva sample.

#### **2.4.1 Heart rate variability data**

Selected morning and evening resting ECG samples were visually inspected to ensure the recordings were free of muscle artefact and ectopic beats. The samples recorded using the XLTEK EEG system were exported as text files, the sampling rate used to record the ECG was identified (256, 512, or 1024 Hz), and the files were subsequently manually converted into corresponding time-data series [12, 37]. The HRV parameters were calculated from the data series using the Matlab based Kubios HRV software [57] for Mac (version 2.2, 2014).

#### **2.4.2 Saliva samples**

Saliva samples were centrifuged at 1000 x g for 2 minutes [54]. The supernatant samples obtained were frozen and stored at - 20°C until they were sent for the LC-MS/MS analyses at the Department of Clinical Biochemistry, University Hospital of South Manchester.

### **2.5 Statistical Analyses**

The data were analysed using the Statistical Package for the Social Sciences (SPSS; version 22 for Mac; SPSS Inc., U.S.A.). Before analysis, all measures were checked for normality using the Shapiro-Wilk test. The physiological measures (HRV and cortisol) were non-normally distributed and were therefore normalised using natural log-transformation. A single morning and evening measure for each patient was obtained by calculating each person's mean morning and mean evening measures. The mean measures were compared between the two patient groups using a series of analyses of variance (ANOVA), accounting for the presence of seizures during the vEEG/ECG recording period (i.e., whether or not patients experienced any seizures during the recording): three-way ANOVAs for mixed designs were performed with time of day (morning vs. evening) as a within-subject independent variable, and diagnostic group (epilepsy vs. PNES) and presence of seizures (no seizures vs. at least one seizure during the recording) as between-subjects independent variables. The associations between measures were explored with Pearson's product-

moment correlation analyses, using all available data points from all patients. In view of the fact that this was an exploratory study, two-tailed  $p$ -values of  $< .05$  were considered statistically significant in the primary analyses [58]. In order to adjust for multiple comparisons, Bonferroni correction was applied to the results in an additional secondary analysis.

### **3. Results**

#### **3.1 Participants**

Of 113 patients approached to participate in the study, a total of 55 patients were recruited. Twenty two patients received a diagnosis of epilepsy (13 females, 59.1%); of those, 15 patients had a diagnosis of documented epilepsy based on a video-EEG recording of a typical seizure, in the remaining seven patients the diagnosis was established through expert clinical assessment by two epilepsy specialists. A diagnosis of PNES was established for 23 patients (eight females, 34.8%). Seventeen patients had a documented diagnosis of PNES confirmed by video-EEG, the remaining six patients had a clinically established diagnosis based on expert clinical consensus. A further five patients were diagnosed as having a mixed disorder (all females, 100%) and in five patients the diagnosis remained uncertain (four females, 80%). Patients with mixed or uncertain diagnosis were excluded from the analyses.

The demographic and clinical characteristics of the 45 patients and the baseline comparisons between patients with epilepsy and those with PNES are shown in Table 1. Of the 22 epilepsy patients, eight (36.4%) were surgery candidates; the remaining 14 had been admitted for diagnostic video-telemetry.

----- Insert Table 1 here -----

#### **3.2 Diurnal variability of daily stress measures**

The morning and evening measures in the two groups are presented in Table 2.

----- Insert Table 2 here -----

##### **3.2.1 Self-reported stress**

The ANOVA showed there were no significant main effects of group, time, or presence of seizures during the recording period, and no significant interactions for self-reported stress ( $p$ 's  $> .05$ ), suggesting that the mean levels of self-reported stress were similar in the mornings and evenings, comparable in patients with epilepsy and those with PNES, and unrelated to whether seizures had been observed during the study period (Figure 1).

##### **3.2.2 Salivary cortisol**

Examination of the morning and evening cortisol showed there were no significant main effects of patient group and no significant main effects of presence of seizures ( $p$ 's  $> .05$ ); however, there was a significant main effect of time of day,  $F(1, 38) = 283.27$ ,  $p < .001$ . Cortisol levels were higher in the morning than in the evening in both patient groups, both in those with and without seizures (Figure 1). No significant interactions were found ( $p$ 's  $> .05$ ).

A further two-way ANOVA showed no significant difference in cortisol deltas between patients with epilepsy and those with PNES,  $F(1, 38) = 0.04$ ,  $p = .836$ , or between those who



experienced seizures during the recording period and those who did not,  $F(1, 38) = 1.32$ ,  $p = .259$ , and there was no significant interaction, suggesting the diurnal cortisol changes were similar in the two patient groups and in those with and without seizures.

### **3.2.3 Heart-rate variability parameters**

There was a significant main effect of patient group on CSI,  $F(1, 33) = 6.59$ ,  $p = .015$ . CSI was higher in patients with PNES than in those with epilepsy. The analysis also revealed a significant main effect of time of day on CSI,  $F(1, 33) = 9.07$ ,  $p = .005$ . Overall, CSI was higher in the morning than in the evening (Figure 1). There were no significant main effects of seizures and no significant interactions ( $p$ 's  $> .05$ ).

No significant main effects or interactions were found for SDNN, RMSSD or CVI ( $p$ 's  $> .05$ ), suggesting there was no difference between patients with epilepsy and those with PNES, no difference between patients with and without seizures, and no difference between the morning and evening values of SDNN, RMSSD or CVI.

----- Insert Figure 1 here -----

### **3.2.4 Correcting for multiple comparisons**

Applying a Bonferroni correction for multiple comparisons to the seven ANOVA tests performed leads to an adjusted significance level of  $p = .007$  ( $.05/7$ ). The reported effects therefore remain statistically significant, with the exception of the main effect of patient group on the heart rate variability parameter CSI. With the Bonferroni-corrected significance level, the difference in the CSI levels between patients with PNES and patients with epilepsy is no longer statistically significant.

## **3.3 Associations between daily measures**

Examination of the correlations between the morning and evening self-reported stress, cortisol and HRV showed a significant negative correlation between the morning salivary cortisol and SDNN in patients with epilepsy;  $r(38) = -.349$ ,  $p = .027$  (Table 3a). Higher morning cortisol levels were associated with lower overall heart-rate variability. There were no significant correlations between self-reported stress and any of the physiological measures, including cortisol and HRV ( $p$ 's  $> .05$ ).

Correlations between the evening measures are shown in Table 3b. No significant correlations were found between self-reported stress and cortisol or between self-reported stress and any of the HRV measures. Nor were there any significant correlations between cortisol and HRV ( $p$ 's  $> .05$ ).

----- Insert Table 3 here -----

As shown in Table 4, there were no significant associations between any of the morning or evening stress measures in patients with PNES ( $p$ 's  $> .05$ ). However, it is worth noting that most of the morning and evening HRV parameters were significantly correlated with each other in both patients with epilepsy and those with PNES ( $p$ 's  $< .05$ ).

----- Insert Table 4 here -----

#### **4. Discussion**

This prospective exploratory study was intended to capture a range of psychological and physiological stress measures in patients with epilepsy and those with PNES, to describe the daily patterns of these measures and their associations with each other.

Although we did find correlations between the two physiological measures of stress used in this study (HRV and cortisol), we found no relationship between these measures and self-reported stress. Furthermore, we only detected relatively few differences in the correlation patterns between the epilepsy and PNES groups.

Examination of the physiological stress measures showed a diurnal pattern with higher levels of physiological arousal in the mornings and lower levels at night in both patients with epilepsy and those with PNES, regardless of whether seizures had been captured during the monitoring period or not. Similarly to findings from healthy individuals [21], salivary cortisol levels were high in the mornings and almost undetectable in the evening in the two patient groups studied. In keeping with the diurnal pattern reflected by the levels of salivary cortisol, the HRV parameter reflecting sympathetic nervous system tone (CSI) also showed higher values in the morning and was lower in the evening. In this study, the sympathetic metric was also higher overall in patients with PNES than in those with epilepsy. This is different from the findings of Ponnusamy [37] who observed no significant differences in resting parasympathetic or sympathetic HRV parameters between patients with epilepsy and those with PNES (although both differed from healthy controls). However, it is worth noting that the difference in CSI between the two patient groups in the present study lost its statistical significance after correction for multiple comparisons. In terms of the circadian patterns of HRV, a day-night pattern has been reported in healthy individuals, with a peak of the vagal tone at night, a decrease towards a sympathetic dominance in the morning and a plateau throughout the day [59].

In contrast to the results of the study by Ronkainen and colleagues, which found suppressed circadian HRV in patients with epilepsy [35], the results of the present study suggest that the circadian changes in both patients with epilepsy and patients with PNES follow the normal day – night pattern, at least in terms of sympathetic nervous system tone. A clear diurnal fluctuation of HRV was also reported in a doctoral thesis on the autonomic function in epilepsy, which assessed 24-hour HRV in 66 patients with intractable epilepsy and found a pattern with high vagal tone (RMSSD) at night and lower vagal tone in the morning and throughout the day but no significant differences in the overall HRV (SDNN) between day and night time [60]. In contrast to the findings described in that thesis, the present study detected a diurnal fluctuation of sympathetic nervous system tone rather than vagal tone. Having said that, neither the present study nor the PhD thesis involved a control group of healthy participants and it is therefore possible that the differences between the morning and evening HRV in the patient samples are not as pronounced as those seen in healthy individuals and/or that HRV in the patient groups may be reduced overall.

We found a significant negative relationship between morning HRV and cortisol in patients with epilepsy but not in patients with PNES. The fact that no equivalent relationships were found between the evening measures is likely to be explained by the lack of inter-subject variability of the (physiologically) low cortisol levels in the evenings. Previous data on the relationship between the HPA axis and the HRV are limited. A study of a group of healthy psychology students captured a significant negative association between cortisol awakening

response and resting HRV later in the day but no associations between awakening-induced changes in cortisol and awakening-induced changes in HRV [61]. A study in healthy medical students failed to show a correlation between cortisol and HRV at rest but identified a negative correlation under stressful conditions on an examination day [62]. Similarly, a study of healthy nurses working shifts suggested that the two systems might function relatively independently during everyday situations characterised by low levels of stress but that cortisol and HRV responses become more closely correlated in highly stressful situations [63]. The findings of a correlation of morning HRV and cortisol observed in the epilepsy group in the present study is therefore more in keeping with the correlational pattern found in stressed than non-stressed healthy individuals.

The diurnal pattern of the HRV and cortisol measures with higher physiological arousal in the mornings and lower arousal in the evening was not reflected in the subjectively reported levels of stress, suggesting that there is significant discrepancy between objective physiological measures and subjective self-reported measures. This discrepancy has been noted in other studies. For example, in Stalder et al [61], cortisol, heart rate and HRV were not associated with self-reported measures of either perceived stress or emotional regulation in a group of psychology students. Furthermore, both patients with epilepsy and patients with PNES tend to have relatively high levels of alexithymia (i.e., difficulty in identifying, understanding and describing own emotions), which may cause further inaccuracies in their self-reports [64-66]. This highlights the complexity of the experience of stress and the difficulty of its assessment in patients with seizures, as patients' subjective perceptions may not match or reliably reflect underlying physiological processes. However, it is also possible that self-report stress measures, particularly the momentary version of the SSSI questionnaire used in the present study, are better at capturing acute change in stress levels than more persistent stress-related arousal. One example of an acute stressor may be the experience of seizures during the video-EEG/ECG; however while we accounted for the presence of seizures during the study period, the morning and evening stress measurements were mostly conducted during the inter-ictal resting state.

#### **4.1 Limitations**

The study has a number of limitations. The main limitations are related to its exploratory nature and its correlational design, which cannot establish causal relationships between the variables. Furthermore, although the sample size is comparable to that of similar previous studies, only a relatively limited number of data-points were available for analysis. The findings of this study therefore need to be interpreted with caution. It would have been ideal to study the physiological parameters around the clock, which would have enabled us to, for example, analyse cortisol day curves. In fact, as data were only collected in the morning and in the evening, it may be more appropriate to consider our study a project looking at morning versus evening comparisons. However, for the sake of brevity, we have used the term 'diurnal' patterns.

It would have also been interesting to consider additional factors with potential effects on the different stress measures, such as sleep duration, sleep deprivation, nature or timing of different antiepileptic medications. Anti-epileptic drugs may affect both salivary cortisol levels and HRV measures [25, 68]. However, in view of the limited sample size of our study and the fact that the analyses were already complex we did not feel that we could accommodate any additional data in our analyses. Furthermore, there is currently no literature available to provide comprehensive guidance on how to correct for the effects of particular AEDs.

It is possible that some individuals may experience the hospital environment as well as the daily diagnostic procedures, changes in medication, altered sleep duration and quality or the anticipation of seizure occurrence as stressful. On the other hand, being away from the context of common everyday demands and hassles and stressful or dysfunctional family relationships and interactions may reduce stress for some patients. This means that the levels of stress captured in this study may have differed from those in patients' natural home environment. Indeed, the mean baseline self-reported stress score in our epilepsy patients appears lower than that reported by Thapar et al. [40]. In their study, patients were recruited from general practices and may have therefore been under less stress and/or less likely to suffer from severe or refractory epilepsy.

In addition, of those we approached to participate in the study, 48.7% consented to participate. It would have been interesting to examine the levels of stress, anxiety, depression and other parameters in those who declined, however we did not have access to information about patients who did not consent to participation.

While we included the presence of seizures during the video-EEG/ECG recording period in the analyses and the results showed no significant differences between patients with and those without seizures, this was only a very crude classification, as some people had more frequent and more severe seizures than others. This classification also did not take account of the temporal relationship between seizures and sampling points. This may be relevant, as previous research suggests that seizures are not distributed randomly throughout the day [67]. However, there were no significant differences in terms of the number of seizures experienced by patients with epilepsy or PNES during the monitoring period and the proportion of people in whom seizures were recorded was also comparable in patients with epilepsy and those with PNES.

## **4.2 Future Research**

This exploratory study highlights the complexity of the experience of stress and the relationship of its different components with each other. Further examination of these relationships is therefore warranted. For example, future studies could include a control group of healthy participants in order to compare the diurnal patterns of physiological stress measures between patients with seizures and healthy individuals. The present study could be replicated with a larger sample of patients and with more data collection points throughout the day. A sufficiently powered study could also perform sub-group analyses to explore factors that may make certain sub-groups of patients particularly vulnerable to the effects of stress (such as past experience of trauma), or measure factors with potential effects on patients' resilience to stress. In those with epilepsy, larger studies could take account of focus localisation or the relationship between the timing of seizures and sampling times. In order to improve the ecological validity of the findings, it would also be interesting to replicate this study in an outpatient sample.

## **4.3 Conclusions**

Despite the limitations of the study, the present findings offer new insights into the interrelationships between different modalities of stress in patients with seizures and contribute to previous studies of the diurnal patterns of physiological stress measures in this population. Based on the finding of a diurnal pattern of both cortisol and HRV found in this study, future cortisol and HRV-based studies in patients with seizures will need to take into

consideration the time of day when the study was conducted. Importantly, the results of the present study indicate that there is a discrepancy between patients' physiological responses and their subjective perceptions, suggesting that simple self-reports cannot be used as a proxy of physiological arousal in patients with seizures and stress. Stress in these patient groups should therefore be studied using a combination of complementary measures.

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